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Paracetamol Is Associated with a Lower Risk of COVID-19 Infection and Decreased ACE2 Protein Expression: A Retrospective Analysis

Nuno Santos Leal †, Yizhou Yu †, Yuwen Chen, Giorgio Fedele and Luís Miguel Martins *

MRC Toxicology Unit, Gleeson Building, Tennis Court Road, University of Cambridge, Cambridge CB2 1QR, UK; njs76@mrc-tox.cam.ac.uk (N.S.L.); yzy21@cam.ac.uk (Y.Y.); yc487@cam.ac.uk (Y.C.); gf334@mrc-tox.cam.ac.uk (G.F.)

* Correspondence: martins.lmiguel@gmail.com

† These authors contributed equally to this work.

Abstract: Ibuprofen is a common over-the-counter drug taken for pain relief. However, recent studies have raised concerns about its potential toxic effect with coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has been proposed that ibuprofen may increase levels of angiotensin-converting enzyme 2 (ACE2), the human receptor for SARS-CoV-2 infection. Therefore, paracetamol is suggested as an alternative to ibuprofen for treating COVID-19 symptoms. Nevertheless, the relationship between intake of paracetamol or ibuprofen and either susceptibility to infection by SARS-CoV-2 or modulation of cellular ACE2 levels remains unclear. In this study, we combined data from human medical records and cells in culture to explore the role of the intake of these drugs in COVID-19. Although ibuprofen did not influence COVID-19 infectivity or ACE2 levels, paracetamol intake was associated with a lower occurrence of COVID-19 in our cohort. We also found that paracetamol led to decreased ACE2 protein levels in cultured cells. Our work identifies a putative protective effect of paracetamol against SARS-CoV-2 infection. Future work should explore the molecular mechanisms underlying the relationship between paracetamol and COVID-19.

Keywords: paracetamol; acetaminophen; ibuprofen; ACE2; COVID-19; SARS-CoV-2; UK Biobank

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1. Introduction

Due to their analgesic and antipyretic properties, ibuprofen [(2RS)-1[4-(2-methyl propyl) phenyl] propionic acid (BP. 2004)] and paracetamol (also known as acetaminophen or N-acetyl-para-aminophenol) are the most frequently used over-the-counter drugs to relieve pain [1–3]. However, these drugs show different adverse effects. Ibuprofen has been shown to affect the gastrointestinal and cardiac systems while paracetamol is often used as an alternative for patients with issues in these systems [3]. Accordingly, these drugs are being used in treatment for coronavirus disease 2019 (COVID-19), a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus can be transmitted via several routes, including respiratory droplets, direct contact with contaminated surfaces, and faecal–oral transmission [4]. The molecular mechanisms that allow the entry of SARS-CoV-2 into human cells include binding of the viral spike (S) protein to the human receptor angiotensin-converting enzyme 2 (ACE2) and S cleavage by the host transmembrane protease serine 2 (TMPRSS2) [4,5]. ACE2, together with its homologue ACE, regulates the renin–angiotensin–aldosterone system, which is involved in regulating blood pressure and electrolyte homeostasis. Angiotensinogen is produced in the liver and cleaved by renin, forming angiotensin I (Ang I). Ang I is then converted to angiotensin II (Ang II) by ACE, and ACE2 cleaves Ang II to angiotensin (1–7). Ang II has vasoconstrictive, pro-inflammatory, and pro-oxidative effects, whereas angiotensin 1–7 elicit vasodilating, anti-inflammatory, and antioxidant responses [6,7].

Circa March 2020, French authorities reported the possibility of negative effects of ibuprofen in COVID-19 patients through the modulation of ACE2 levels [8–10]. The concern was based on findings that patients with hypertension and diabetes mellitus were at an increased risk of COVID-19, possibly due to treatment with ACE inhibitors and angiotensin II type I receptor blockers, which leads to increased levels of ACE2 [11]. Additionally, ibuprofen reportedly increased ACE2 levels in a rat model of type 1 diabetes [9,12]. Therefore, paracetamol has been suggested as an alternative to ibuprofen for treating pain and high temperature in patients with COVID-19 [8,10].

Although a few studies have shown that ibuprofen intake does not worsen the clinical outcome of COVID-19 [13], it remains unknown whether ibuprofen and paracetamol influence SARS-CoV-2 infectivity. Moreover, despite ibuprofen-induced increases in ACE2 in rats [12], no human study has assessed whether ibuprofen or paracetamol affects levels of ACE2. The aims of this study are, first, to assess if ibuprofen or paracetamol affect SARS-CoV-2 infectivity, and second, to determine if they modulate ACE2 levels. Using an analysis of half a million individuals that are part of the UK Biobank cohort, we show that those recorded as taking paracetamol, but not ibuprofen, had a lower risk of testing positive for SARS-CoV-2. We also found that treating human Caco-2 cells with paracetamol decreased ACE2 protein levels.

2. Materials and Methods

2.1. UK Biobank Data Sources

The UK Biobank contains health data from over 500,000 community volunteers based in England, Scotland, and Wales. Information about the geographical regions, recruitment, and other characteristics has been previously reported [14]. Briefly, between 2006 and 2010, adults aged between 40 and 69 years were invited to participate, and extensive demographic, lifestyle, clinical, and radiological information was collected. Baseline assessments included a comprehensive series of questionnaires, face-to-face interviews, physical examinations, and blood sampling, with links to electronic medical records. Clinical data for dementia and other comorbidities were cross validated by an algorithm from the UK Biobank, which considered UK Biobank baseline assessment data (verbal interview), linked hospital admissions data, and death register data.

Data regarding medications are recorded as indicated in Resource 100235 of the UK Biobank. In brief, by using a touchscreen questionnaire under the guidance of a UK Biobank interviewer, the participants reported whether they regularly took any over-the-counter or prescription medications. As regularity was defined as more frequently than every 3 months, short-term medication was excluded. We did not include information on the dosage of each medication since these data were not available.

The method of linking COVID-19 results to UK Biobank participants has been previously published [15]. Briefly, Public Health England's Second-Generation Surveillance System is a centralised microbiology database covering English clinical diagnostics laboratories that provides national surveillance of legally notifiable infections, bacterial isolations, and antimicrobial resistance. Public Health England issues a regular feed of COVID-19 test results to the UK Biobank using a secure dynamic linkage algorithm.

Ethical approval from the UK Biobank was granted from the North West Multi-Centre Research Ethics Committee. The current analysis was approved under UK Biobank application #60124. A detailed list of variables evaluated in the present study is presented in Table S1. We defined high blood pressure using the criterion of diastolic blood pressure $\geq$90 mmHg or systolic blood pressure $\geq$140 mmHg. Individual-level data were collected from the UK Biobank on 5 February 2021.

2.2. Cell Culture and Drug Treatments

A549 cells were grown in Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA, 41966) supplemented with 10% foetal bovine serum (FBS; Merck, Kenilworth, NJ, USA, F9665); Calu-3 cells were grown in DMEM/F-12 medium

(Thermo Fisher Scientific, Waltham, MA, USA, 11330-032) supplemented with 15% FBS and Caco-2 cells in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA, USA, A10491-01) supplemented with 20% FBS. All cell media were supplemented with 1% penicillin and streptomycin (Thermo Fisher Scientific, Waltham, MA, USA, 15070063). The cells were split, and the media renewed every 2–3 days. The cells were seeded in 6-well plates at a density of 175,000 (A549 and Caco-2) or 400,000 (Calu-3) cells per well for 24 h and then treated or lysed. Caco-2 cells were treated with paracetamol (Merck, Kenilworth, NJ, USA, P0300000) (0.1, 0.5, 1, 2.5, and 5 mM) or ibuprofen (Merck, Kenilworth, NJ, USA, I4883) (0.05 mM, 0.25 mM, 0.5 mM, 1 mM, and 2 mM) for 24 h. Dimethylsulfoxide (DMSO; 0.02%, Merck, Kenilworth, NJ, USA, 276855) was added as a control for ibuprofen. Drugs were directly added to the cell media. All cells tested negative for mycoplasma. Cell lines were obtained from Professor Anne Willis's group. A549 cells are derived from human epithelial lung carcinoma cells, Caco-2 cells are derived from human epithelial colorectal adenocarcinoma cells, and Calu-3 are derived from human epithelial lung adenocarcinoma cells. The Caco-2 cell line was validated by STR genotyping while the other cells were not validated. Drug concentrations were chosen based on previously published articles reporting changes in processes in different cell lines and, when possible, specifically in Caco-2 cells, as follows: paracetamol [16–20] and ibuprofen [21–25].

2.3. Protein Extraction and Western Blotting

Protein extracts from cells treated as described were prepared by lysing cells in RIPA digestion solution [150 mM NaCl (Fisher Chemical, Waltham, MA, USA, S/3161/53), 1% Triton X-100 (BDH, Poole, UK, 306324N), 0.5% sodium deoxycholate (Sigma, St. Louis, MI, USA, D5670), 0.1% SDS (Sigma, St. Louis, MI, USA, 05030), 50 mM Tris pH = 7.5 (Fisher BioReagents, BP152-1)] with 1× proteinase inhibitors cOmplete Mini, EDTA-free (Roche, Basel, Switzerland, 11836170001), and benzonase solution (50 mM Tris pH = 8.0, 4 mM MgSO4 (Scientific Laboratory Supplies, Nottingham, UK, CHE2456) and 1× benzonase (EMD Millipore, Burlington, MA, USA, 70664)). Protein concentration was measured using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA, 23227). All extracts were mixed with 4 × LDS loading buffer. For sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), equivalent amounts of proteins were resolved on a 4–12% Bis-Tris gel (Thermo Fisher Scientific, Waltham, MA, USA, NP0335BOX and NP0336BOX). Proteins were then transferred to nitrocellulose membranes and the membranes were blocked in Tris-buffered saline (TBS; 0.15 M NaCl and 10 mM Tris, pH 7.5) with Tween 20 (Sigma, St. Louis, MI, USA, P1379) (TBS-T) containing 5% (*w*/*v*) dried non-fat skim milk (BD, 232100) for 1 h at room temperature and then probed with the primary antibody before being incubated with the appropriate IRDye-conjugated secondary antibody donkey anti-mouse (LI-COR, Cambridge, UK, 926-32212, Lot: C91023-09) or donkey anti-rabbit (LICOR, Cambridge, UK, 926-32213, Lot: C91112-09). Antibody complexes were visualised using an Odyssey (LI-COR, Cambridge, UK,), and quantifications were performed using Image Studio Lite version 5.2.5 (LI-COR, Cambridge, UK,), with normalisation to the respective loading control (tubulin). Mouse monoclonal anti-α-tubulin (Merck, Kenilworth, NJ, USA, T6074, RRDI: AB_477582, clone number: B-5-1-2, lot: 034M4837) and rabbit monoclonal anti-ACE2 (Abcam, ab108252, RRDI: AB_10864415, clone number: EPR4435(2), lot: GR3344245-2) antibodies were used. Both primary antibodies were used at 1:1000 dilution and secondary antibodies at 1:20,000 dilution. Antibody validation can be found on the companies' websites as well as the references for these validations.

2.4. RNA Extraction and Quantitative Real-Time PCR

After 24 h of drug treatment, cells were washed 1× with phosphate-buffered saline (PBS), trypsinised and centrifuged for 5 min at 500× *g*. The trypsin was removed, and total RNA was extracted using TRIzol (Life Technologies, Carlsbad, CA, USA, 15596018) and quantified by spectrophotometric analysis using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA,). Quantitative real-time PCR with reverse

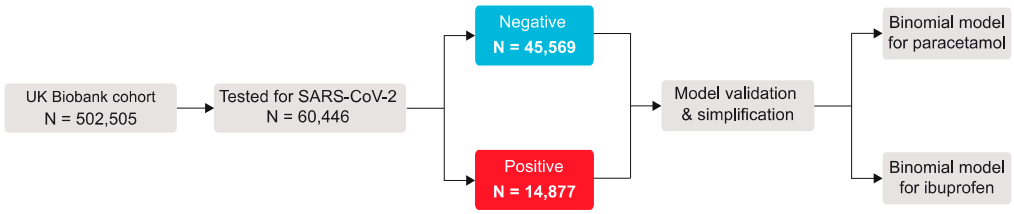
transcription (qRT-PCR) was performed using a real-time 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) with a SensiFAST SYBR Lo-ROX One-Step Kit (Bioline, London, UK, BIO-74005). Fold change was calculated using the comparative CT method [26]. For qRT-PCR, we measured the coefficient of variation (CV) of the technical replicates and excluded from statistical analysis samples with a CV over 3%. Gene-specific primers were obtained from Sigma, St. Louis, MI, USA: ACE2—forward, 5′-CGAAGCCGAAGACCTGTTCTA-3′ and 5′-CAAGAGCAAACGGTTGAACAC-3′, and reverse, 5′-GGGCAAGTGTGGACTGTTCC-3′ and 5′-CCAGAGCCTCTCATTGTAGTCT-3′ [27]; GAPDH—forward, 5′-CTGACTTCAACAGCGACACC-3′, and reverse, 5′-TAGCCA AATTCGTTGTCATACC-3′). GAPDH was used as a housekeeping gene.

2.5. Statistical Analysis

For the UK Biobank analysis (Figure 1), we first employed an exploratory approach to identify which putative comorbidities influence the risk of testing positive for SARS-CoV-2. We obtained a list of 18 variables (Table S1) based on 3 publications that explored risk factors influencing SARS-CoV-2 infection [28–30]. We then applied an iterative variable selection procedure combining unsupervised stepwise forward and stepwise backward regression analyses to select the most suitable predictor or combination of predictors in our models based on the Akaike information criterion. We calculated the proportional odds and their 95% confidence intervals based on the coefficients of the binomial models to quantify the effects of paracetamol and ibuprofen intake on the risk of testing positive for SARS-CoV-2 [31]. The UK Biobank analysis was performed in R version 4.0.0 [32] and Python version 3.7 in Jupyter Notebook version 5.5 [33]. The analysis source code, detailed quality checks, and all supplementary material are available on GitHub (https://github.com/M1gus/NSAIDs_Ace2) (Accessed on 12 August 2021).

For Figure 2, the Kruskal–Wallis test with correction using Dunn's multiple comparison test was utilised to test for significance, as the data did not follow a normal distribution based on the Shapiro–Wilk test. For samples treated with drugs (Figures 3 and 4), samples were normalised to their respective control and found to be normally distributed based on the Shapiro–Wilk test. We performed a one-sample *t*-test followed by a correction to control for the false discovery rate (FDR) using the two-stage step-up method of Benjamin, Krieger, and Yekutieli at a 5%. Analyses of the mRNA and protein levels were done in GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as a p value or corrected p value ≤ 0.05. ** $p < 0.01$, * $p < 0.05$.

A



B

	Negative	Positive
Drug intake		
Paracetamol	52,789 (12.2%)	87 (10.3%)
Ibuprofen	6071 (13.3%)	2206 (14.8%)
Physiological health factors		
Pre-existing diagnosis of dementia	303 (0.7%)	151 (1.0%)
Waist-to-hip ratio	0.88 ± 0.09	0.88 ± 0.09
Age	69.73 ± 8.00	65.10 ± 4.39
Male	21,065 (46.2%)	7,032 (47.3%)
Female	24,504 (53.8%)	7,845 (52.7%)
Number of cancers	0.12 ± 0.25	0.08 ± 0.29
Pre-existing diagnosis of COPD	227 (0.5%)	45 (0.3%)
Education		
College or University degree	5086 (11.4%)	1305 (9.0%)
Professional qualifications	12,451 (27.9%)	3618 (24.9%)
NVQ/HND/HNC	6275 (14.1%)	2607 (17.9%)
CSEs	2673 (6.0%)	1498 (10.3%)
O levels/GCSEs	8084 (18.1%)	2327 (16.0%)
None of the above	8715 (19.5%)	2823 (19.4%)
A levels or equivalent	1321 (3.0%)	376 (2.6%)
Social health determinants		
White British ethnicity	40,112 (88.0%)	12,444 (83.6%)
Minority ethnicity	5457 (12.0%)	2433 (16.4%)
Townsend deprivation index	−1.24 ± 3.14	−0.70 ± 3.26
Number of people in the household	2.32 ± 1.38	2.73 ± 1.49
Flat or appartment	4370 (9.6%)	1473 (9.9%)
House	40,632 (89.5%)	13,242 (89.3%)
Care home	17 (0.0%)	6 (0.0%)
Temporary structure	79 (0.2%)	11 (0.1%)
Sheltered accommodation	180 (0.4%)	51 (0.3%)
None of the above type of housing	141 (0.3%)	44 (0.3%)

Figure 1. Paracetamol intake is associated with a lower risk of SARS-CoV-2 infection. (**A**) Workflow of the analysis. (**B**) Descriptive statistics of the cohort analysed relative to whether SARS-CoV-2 infection occurred. For continuous variables such as age, the average age and standard deviation are shown; for discrete variables such as sex, the corresponding number of UK Biobank participants and their percentage relative to the total cohort are indicated.

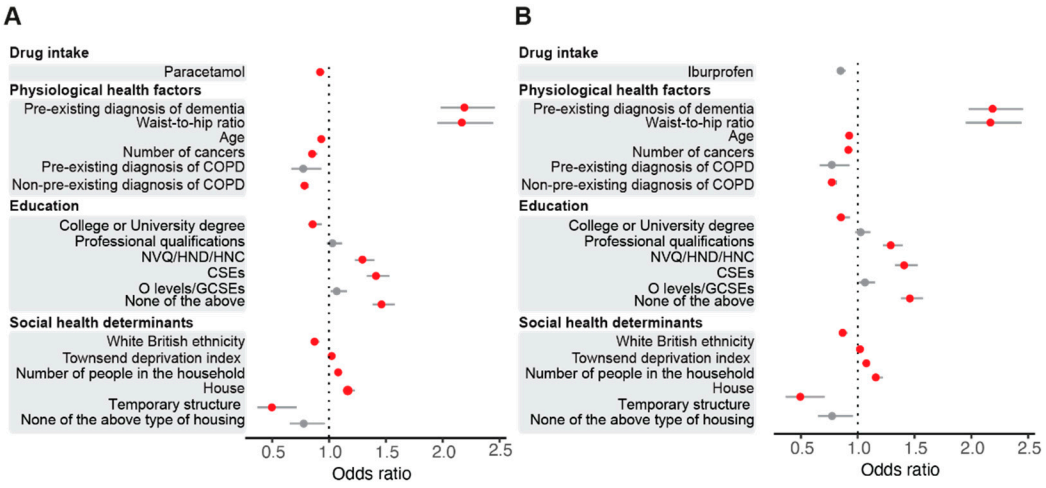


Figure 2. Paracetamol intake is associated with a lower risk of SARS-CoV-2 infection. (**A**) Relationship between paracetamol intake and SARS-CoV-2 infection. The numeric values of the odds ratios are shown in Table S2. (**B**) Relationship between ibuprofen intake and SARS-CoV-2 infection. The numeric values of the odds ratios are shown in Table S3. For both figures, the odds ratios calculated from logistic regressions and their respective 95% confidence intervals of the relationship, as well as covariates, are provided in Supplementary Tables. Statistically significant (p value ≤ 0.05) covariates are shown in red and non-significant (p value > 0.05) covariates in grey.

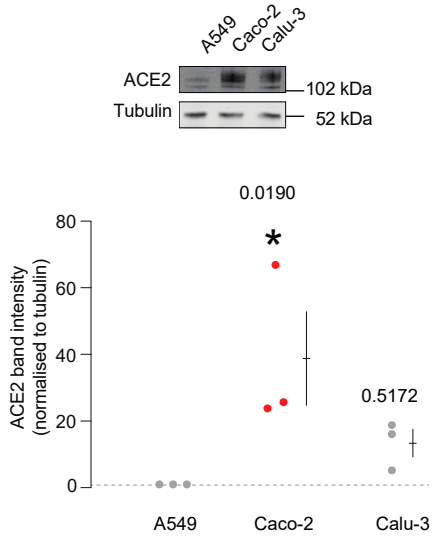


Figure 3. Levels of ACE2 in A549, Caco-2, and Calu-3 cell lines. Quantification of ACE2 protein levels normalised to tubulin in A549, Caco-2, and Calu-3 cell lines. Descriptive statistics (mean and standard error of the mean, s.e.m.) are shown to the right of the individual values for each data set. Three biologically independent samples were used per cell line. Significance is shown with an asterisk and red; non-significant p values are also shown (the Kruskal–Wallis test with correction using Dunn's multiple comparison test since data did not follow normal distribution).

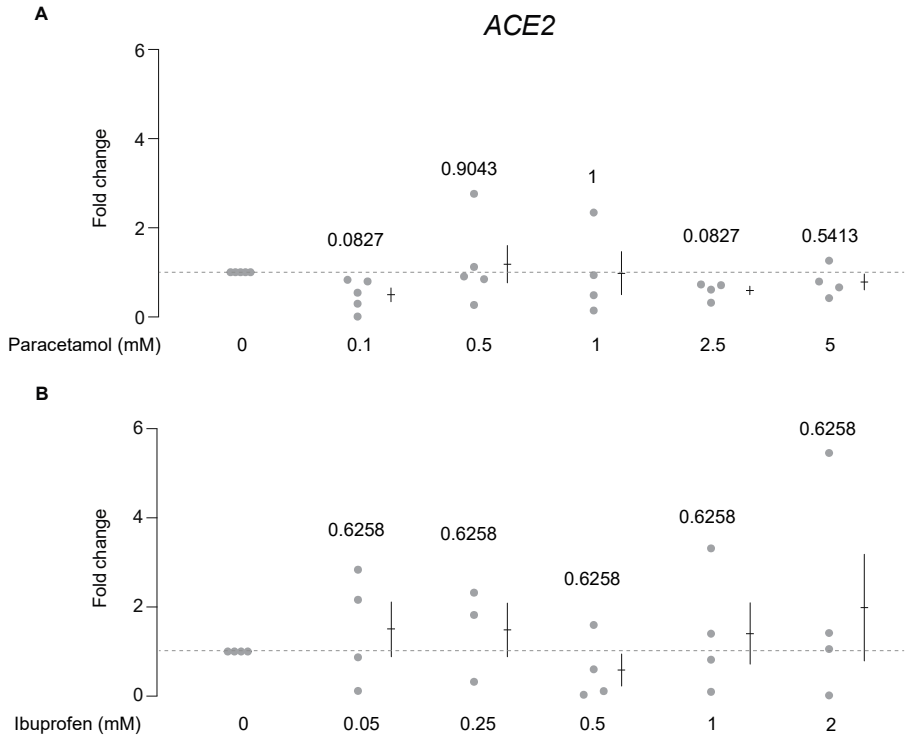


Figure 4. Paracetamol and ibuprofen do not influence ACE2 mRNA levels in Caco-2 cells. Quantification of ACE2 mRNA levels in Caco-2 cells after treatment with (**A**) paracetamol or (**B**) ibuprofen for 24 h. Descriptive statistics (mean and s.e.m.) are shown to the right of the individual values for each data set. Four or five biologically independent samples were used per condition. Non-significant corrected *p* values are shown (one-sample *t*-test with correction using the two-stage step-up method of Benjamin, Krieger, and Yekutieli for FDR correction at 5%).

3. Results

3.1. Regular Paracetamol Intake Results in a Lower Risk of SARS-CoV-2 Infection in the UK Biobank Cohort

We used data from the UK Biobank cohort to explore the link between paracetamol and ibuprofen intake in SARS-CoV-2 infection. The UK Biobank is a biomedical database and research resource containing health records for more than half a million UK individuals [34]. From 16 March 2020 to 1 February 2021, 60,446 of 502,505 UK Biobank participants were tested for SARS-CoV-2 (Figure 1A,B); of these, 14,877 were positive. To identify the factors influencing the risk of SARS-CoV-2 infection, we applied an iterative unsupervised machine learning method based on several variables identified previously by us and others [28–30] (Table S1). We found that age, obesity (waist-to-hip ratio), sex, a previous history of cancer, diagnoses of dementia and chronic obstructive pulmonary disorder, ethnicity, deprivation (Townsend deprivation index), education, the number of people in the household, and house type had a significant association with a positive test. We therefore accounted for all these significant variables in all subsequent models.

We next modelled whether previous intake of paracetamol modulates the risk of testing positive for SARS-CoV-2 by using binomial regression and found that paracetamol led to a 6.63% reduction in the risk of infection (odds ratio, OR 0.93; 95% confidence interval (CI) 0.91 to 0.96, *p* value = 0.004) (Figure 2A and Table S2). We applied the same method to assess whether ibuprofen also affects the risk of SARS-CoV-2 infection. Although we found that previous intake of ibuprofen was associated with a 2.98% decrease in the risk of infection (Figure 2B and Table S3), this association was not significant (OR 0.97; 95% CI 0.94 to 1.00, *p* value = 0.29). Taken together, paracetamol, but not ibuprofen, is associated with a lower risk of SARS-CoV-2 infection.

3.2. Paracetamol, but Not Ibuprofen, Decreases ACE2 Protein Levels

As SARS-CoV-2 uses ACE2 on the surface of human cells for infection [5], we examined whether paracetamol alters the risk of infection by SARS-CoV-2 by modulating ACE2 levels. Human Calu-3 and Caco-2 cell lines have been shown to be highly susceptible to infection by SARS-CoV-2 pseudo-virus [5], though the levels of ACE2 in these cell lines have not been reported. Thus, we began by analysing levels of ACE2 protein in these cells; the lung adenocarcinoma cell line A549 was used as a control because it has been shown to be more resistant to infection by SARS-CoV-2 pseudo-virus [5]. Overall, levels of ACE2 in Caco-2 (p value = 0.0190) and Calu-3 (p value = 0.5172, non-significant) cells were higher than those in A549 cells (Figure 3). Therefore, we decided to use Caco-2 cells for our next set of experiments.

Next, we treated Caco-2 cells with paracetamol (concentration range between 0.1 and 5 mM) or ibuprofen (concentration range between 0.05 and 2 mM) for 24 h and assessed levels of ACE2 mRNA and protein by real-time qPCR and Western blotting, respectively. While levels of ACE2 mRNA were not significantly changed in both paracetamol- and ibuprofen-treated cells, there was a tendency for decrease in ACE2 mRNA upon treatment with 0.1 and 2.5 mM of paracetamol (corrected p values for both concentrations = 0.08) (Figure 4A and Table S4), whereas no differences were observed after treatment with ibuprofen (Figure 4B and Table S4).

Similarly, protein levels of ACE2 were significantly decreased with 0.1 mM, 0.5 mM, 1 mM, and 2.5 mM of paracetamol treatment (Figure 5A and Table S5), with no significant differences with 5 mM of paracetamol nor ibuprofen within the tested range of concentrations (Figure 5B, Table S5).

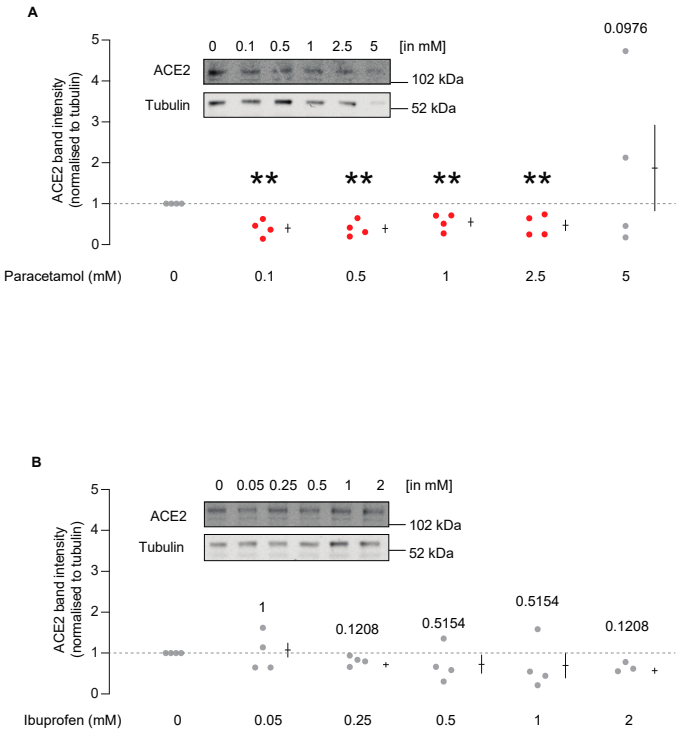


Figure 5. Paracetamol decreases ACE2 protein levels in Caco-2 cells. Quantification of ACE2 protein levels in Caco-2 cells after treatment with (**A**) paracetamol or (**B**) ibuprofen for 24 h. Descriptive statistics (mean and s.e.m.) are shown to the right of the individual values for each data set. Four biologically independent samples were used per condition. Representative cropped immunoblots are shown and the whole immunoblots can be found in Figures S1 and S2. Significance is shown with asterisks and red; non-significant corrected p values are also shown (one-sample t-test with correction using the two-stage step-up method of Benjamin, Krieger, and Yekutieli for FDR correction at 5%).

4. Discussion

The effect of ibuprofen intake and SARS-CoV-2 infection remains unclear and controversial [9,20,35–37]. Additionally, the association between paracetamol, another commonly used over-the-counter pain reliver, and COVID-19 has not been explored. In this study, we combined data from human medical records and in vitro results to investigate the relationship between ibuprofen, paracetamol, and SARS-CoV-2 infection as well as the possible mechanism. We showed that although ibuprofen does not affect SARS-CoV-2 infection in our models, paracetamol is associated with a 6.63% reduction in infection risk. Additionally, we found that the protein levels of ACE2 were decreased in human Caco-2 cells cultured with 0.1 mM, 0.5 mM, 1 mM, and 2.5 mM of paracetamol, while mRNA levels were not significantly changed. Therefore, our data suggest that paracetamol could affect ACE2 protein levels by mechanisms other than alterations in its translation (e.g., protein degradation). Since SARS-CoV-2 enters human cells by binding to ACE2 [5], the decreased levels of ACE2 caused by paracetamol may explain the lower risk of COVID-19 infectivity observed in our model.

Overall, we observed significant variability in ACE2 mRNA levels in cells treated with either paracetamol or ibuprofen (Figure 3). Furthermore, it is unclear why we detected a non-significant decrease in levels of ACE2 in cells treated with 0.1 mM or 2.5 mM paracetamol but not with other dosing regimens (0.5 mM, 1 mM, or 5 mM). In rat hearts, levels of ACE2 mRNA exhibit circadian oscillation [38]. Hence, as we did not account for daily rhythms as a factor in our analysis, it is possible that daily variations in levels of ACE2 in Caco-2 cells account for the variability we observed.

A bulletin published by the UK Commission on Human Medicines and two other studies found no clear evidence that acute use of ibuprofen increases the risk of developing COVID-19 [8,13,39]. Similarly, we did not find evidence that ibuprofen intake alters the risk of SARS-CoV-2 infection or ACE2 levels. Moreover, the data for the regular intake of paracetamol or ibuprofen in UK Biobank participants pre-date the COVID-19 pandemic. Our results suggest that regular uptake of any of these agents possibly modulates ACE2 levels, but experimental validation is required, particularly in animal models. Long-term exposure to paracetamol has been reported to cause epigenetic changes in genes associated with neural development [40], and it is thus important to determine whether the long-term effects of paracetamol on ACE2 have an epigenetic component. Additionally, data regarding the intake of paracetamol by participants during SARS-CoV-2 infection are not available, precluding any conclusion regarding whether the protective effect of paracetamol is due to its intake during viral infection. Although we did not explore the exact molecular pathway by which paracetamol affects SARS-CoV-2 infection, there are several mechanisms that could be behind this, including the ability of paracetamol to reduce the activity of sirtuin 1 [41], a direct activator of ACE2 transcription [42]. Further studies are required to elucidate the mechanisms by which paracetamol alters ACE2 levels.

Additionally, the epidemiological data set analysed in our study has several limitations. Due to the retrospective nature of our methodology, case–control clinical studies are required to elucidate the effect of paracetamol on COVID-19 pathology. Our cohort is predominantly of white ethnicity, which limits the interpretability of our results for people of other ethnicities. Due to limited information from the UK Biobank, we cannot explain the reasons behind the regular uptake of paracetamol or ibuprofen in the participants analysed in our study. Additionally, the duration and dosage of paracetamol or ibuprofen are currently unavailable. COVID-19 deaths are connected with failure in the pulmonary system, such as pneumonia and respiratory failure [43]. However, the Caco-2 cells used in this study are derived from colorectal adenocarcinoma cells and the effect of paracetamol or ibuprofen in ACE2 levels might be different in lung/alveolar cells. Finally, we cannot exclude that the effect of these drugs on ACE2 levels may be altered during infection or in a diseased individual.

5. Conclusions

In this study, we showed that paracetamol is associated with a lower risk of COVID-19 infectivity and a decrease in ACE2 protein levels, while we found no association between ibuprofen and COVID-19 infection.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/covid1010018/s1, Table S1. Detailed descriptive statistics of the analysed cohort; Table S2: Paracetamol intake is associated with a decreased risk of COVID-19 infection; Table S3: Ibuprofen intake does not affect the risk of SARS-CoV-2 infection; Table S4. Individual p values and corrected FDR-corrected p values of Figure 3; Table S5. Individual p values and FDR-corrected p values of Figure 4.

Author Contributions: N.S.L., G.F. and L.M.M. conceived of the original idea. Y.Y. developed and performed the computational modelling. N.S.L. carried out the experimental work with the help of Y.C. and G.F. N.S.L., Y.Y. and L.M.M. wrote the manuscript with the support of Y.C. and G.F. All authors provided critical feedback and approved the final version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. UK Biobank ethical approval was granted from the North West Multi-Centre Research Ethics Committee. The current analysis was approved under UK Biobank application #60124.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study by the UK Biobank.

Data Availability Statement: Access to the UK Biobank data can be applied for via the official UK Biobank website (https://www.ukbiobank.ac.uk/) (Accessed on 5 February 2021). All other analyses can be found in our GitHub repository (https://github.com/M1gus/NSAIDs_Ace2) (Accessed on 12 August 2021).

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Abbreviations

AA	arachidonic acid
ACE	angiotensin-converting enzyme
ACE2	angiotensin-converting enzyme 2
Ang I	angiotensin I
Ang II	angiotensin II
CI	confidence interval
COVID-19	coronavirus disease 2019
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
FBS	foetal bovine serum
mRNA	messenger RNA
NSAIDs	non-steroid anti-inflammatory drugs
OR	odds ratio
qRT-PCR	quantitative real-time PCR with reverse transcription
S	spike
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
s.e.m.	standard error of the mean

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